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Computational Studies on Phosphodiesterase-5 Inhibitors to Design Novel Lead Compounds for the Treatment of Erectile Dysfunction

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Abstract:

2D and 3D Quantitative Structural Activity Relationship studies using Molecular Field Analysis (MFA) and Receptor surface Analysis (RSA) methods along with pharmacophore hypothesis using Catalyst version 4.7 were performed on a series of Phosphodiesterase 5 (PDE-5) inhibitors. The best equations with training set consisting 41 molecules, produced r2 value of 0.788 and r2cv value of 0.618 in 2D-model and r2 value of 0.844 and r2cv value of 0.810 in MFA-model and r2 value of 0.853 & r2cv of 0.799 in the RSA-model. Pharmacophore models were generated using 20 molecules as training set. The best quantitative pharmacophore model consists of one hydrogen bond acceptor, one hydrophobic aliphatic and two ring aromatic features. We have constructed a large set of 75 test compounds, and conformational studies were done as described earlier. The estimated activities were scored using hypothesis 1 as the pharmacophore. Out of 25 highly active compounds (<50nM), 15 were accurately predicted as highly active and the remaining were all predicted as moderately active. Out of the 33 moderately active compounds (50-1000nM), 4 were predicted as inactive and one was predicted highly active. Out of the 16 inactive compounds (>1000nM), 8 were predicted to be inactive and 8 were predicted to be moderately active

Keywords: *3D QSAR, CAT B, MFA, RSA, Catalyst, Pharmacophore.*

Introduction:

A phosphodiesterase is an enzyme that catalyzes the hydrolysis of phosphodiester bonds, for instance a bond in a molecule of cyclic AMP or cyclic GMP. It plays a role in signal transduction by regulating the intracellular concentration of cyclic nucleotides. This phosphodiesterase catalyzes the specific hydrolysis of cGMP to 5'-GMP. Human phosphodiesterase 5 is responsible for the degradation of cyclic GMP in the corpus cavernosum. It is well known target for erectile dysfunction and pulmonary hypertension. The wide-ranging functions of this enzyme therefore make it an attractive drug discovery target [1 and 2].

We have performed Pharmacophore and QSAR studies for developing novel PDE-5 inhibitors [3-8] using the Catalyst 4.7 and Cerius2 program suite respectively [9-29]. QSAR equations has been generated for 51 PDE-5 inhibitors employing Molecular Field Analysis (MFA) as well as Receptor surface Analysis (RSA) using Genetic function approximation (GFA) as regression method. We intend to employ the pharmacophore information to execute 3D-database virtual screening to discover reliable and potential Novel Lead structure against PDE-5 inhibitors for treatment of erectile dysfunction.

Materials and Methods:

All molecular modeling works were carried out by using DISCOVERY STUDIO 2.5 software package (Accelrys, San Diego, CAUSA). [All the catalyst functions are in-built modules of Discovery Studio].

Experimental work:

40 molecules forming the training set were used to generate the QSAR equation. For MFA studies molecular field was created using proton and methyl groups as probes, which represent electrostatic and steric fields respectively. For RSA studies chemical properties namely charge, electrostatic potential, hydrogen bonding propensity and hydrophobicity associated with each surface point were calculated. For generating equations, only 10% of the total descriptors whose variance was highest were considered for further analysis. Regression analysis was carried out using G/PLS method consisting of over 50,000 generations with a population size of 100 (Table. 2.1 and 2.2)

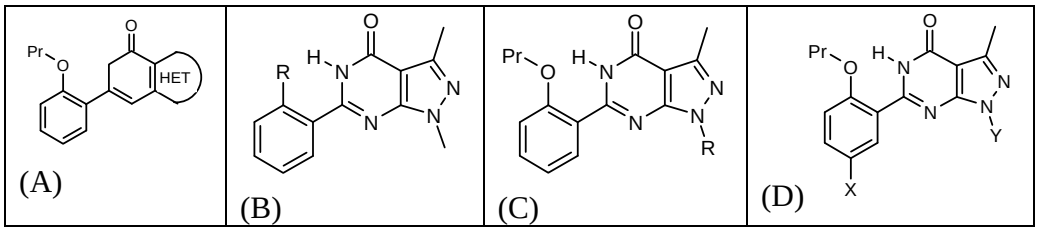
Catalyst version 4.7 was used to generate Pharmacophore models. 20 molecules forming the training set were used to generate Hypogen hypothesis.

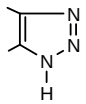
Table 1: Statistical details of 2D, MFA, & RSA analysis

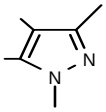
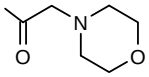
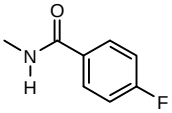
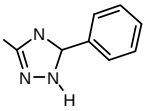
Serial No.	Statistics	2D	MFA	RSA
01	R	0.888	0.919	0.923
02	r^2	0.788	0.844	0.853
03	xvr^2	0.618	0.810	0.799
04	Bsr^2	0.655	0.837	0.846
05	PRESS	10.490	5.227	5.513

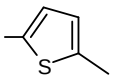
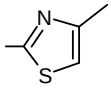
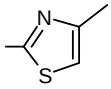
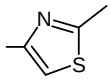
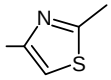
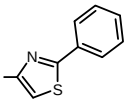
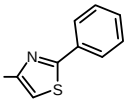
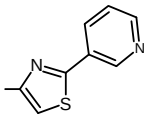
a. MFA: Molecular Field Analysis, b. RSA: Receptor surface Analysis, c. R 2: Regression Analysis, d. XVR2: Cross validated R2, e. PRESS: Predicted sum of squared residuals.

Table 2.1: Training Set with Experimental and Predicted Activity



Compound No.	Scaffold class	HET/R/X	Y	Experimental		Predicted		
				IC_{50} nM	pIC_{50}	2D	MFA	RSA
1	A			200	-2.300	-2.465	-2.219	-2.492
2	A			80	-1.900	-2.174	-2.321	-2.354
3	A			300	-2.480	-2.107	-2.439	-2.535
4	A			70	-1.850	-1.732	-1.898	-1.853
5	A			50	-1.700	-1.814	-1.886	-1.927
6	A			100	-2.000	-2.118	-2.306	-1.921

7	A			60	-1.780	-1.695	-1.586	-1.482
8	A			50	-1.700	-2.023	-1.983	-1.868
9	A			80	-1.900	-1.622	-1.985	-1.570
10	A			8	-0.900	-1.367	-1.249	-0.920
11	A			100	-2.000	-2.001	-1.856	-2.264
12	A			30	-1.480	-1.887	-1.836	-1.840
13	B	O-Isopropyl		40	-1.600	-1.109	-1.249	-1.243
14	C	Butyl		6	-0.780	-0.776	-0.956	-0.868
15	C	Tertiary butyl		20	-1.300	-0.846	-0.957	-1.119
16	C	CH2CF3		7	-0.850	-0.735	-0.956	-1.014
17	D		Me	4	-0.600	-0.823	-0.550	-0.358
18	D	NO2	Me	4	-0.600	-0.983	-0.549	-0.597
19	D	NH2	Et	7	-0.850	-0.678	-0.877	-0.692
20	D	N(Me)2	Me	5	-0.700	-0.918	-0.550	-0.380
21	D	N(Me)2	Et	4	-0.600	-0.928	-0.550	-0.725
22	D	NHSO2Me	Me	3	-0.480	-0.334	-0.551	-0.368
23	D	NHSO2Me	Et	4	-0.600	-0.234	-0.340	-0.528
24	D	NHCOMe	Et	2	-0.300	-0.709	-0.257	-0.503
25	D		Me	2	-0.300	-0.443	-0.550	-0.430
26	D	NHCOOMe	Me	2	-0.300	-0.747	-0.550	-0.477
27	D	NHCONH2	Me	2	-0.300	-0.849	-0.550	-0.577
28	D	NHCONHEt	Me	3	-0.480	-0.545	-0.550	-0.523
29	D	NHCSNHEt	Me	3	-0.480	-0.345	-0.550	-0.545
30	D	NHCSNHCOOEt	Me	2	-0.300	-0.152	-0.550	-0.645
31	D		Me	20	-1.300	-0.387	-0.550	-0.372
32	D		Me	5	-0.700	-0.769	-0.550	-0.732

33	D		Me	4	-0.600	-0.661	-0.550	-0.396
34	D		Me	3	-0.480	-0.882	-0.550	-0.538
35	D		Me	2.5	-0.400	-0.537	-0.551	-0.519
36	D		Et	2	-0.300	-0.412	-0.257	-0.468
37	D		Me	2.5	-0.400	-0.513	-0.551	-0.566
38	D		Et	3	-0.480	-0.454	-0.238	-0.496
39	D		Me	2.5	-0.400	-0.322	-0.550	-0.568
40	D		Et	3	-0.480	-0.316	-0.257	-0.440
41	D		Et	2	-0.300	-0.233	-0.257	-0.437

Et denotes Ethyl, Me denotes Methyl, Pr denotes Propyl

All structures were built and minimized within the Catalyst software package, and conformational analysis of each molecule was implemented using the poling algorithm. Hypotheses were generated from a collection of conformational models of compounds spanning activities of 4-5 orders of magnitude.

Results and discussion:

Molecular field analysis (MFA)

2D equation:

Activity = 32.1973 + 0.11033* “MW” + 0.036525* “Area” –0.163124* “VM” – 31.2449* “Density”

The term MW +0.11033 denotes the molecular volume and the term Vm – 0.163124 denotes the molecular volume

MFA equation:

Activity = -2.57287 + 0.009541* “CH3/549” + 0.022934* “CH3/276” + 0.020199 * “CH3/534” + 0.02451* “CH3/771”

MFA equation that for the probe point of CH3 at position 534 in MFA grid indicates bulky groups are favored to decrease the activity. Stereo view of MFA grid is shown in Fig. 1

Receptor surface analysis (RSA)

RSA equation:

Activity_1 = -1.08728 + 1.35507* “VDW/3789” -2.1011* “ELE/2083” + 3.04312* “ELE/2937” – 1.57952* “VDW/3091”

The term ELE/2083 in the RSA equation indicates electronegative groups are favored to enhance the activity.

Table 2.2: Test Set with Experimental and Predicted Activity

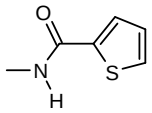
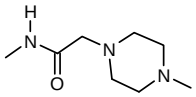
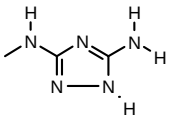
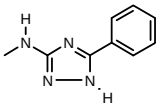
Compound No.	Scaffold class	HET/R/X	Y	Experimental		Predicted		
				IC_{50} (nM)	pIC_{50}	2D	MFA	RSA
1	A		_	200	-2.18	-2.025	-2.110	-1.781
2	B	OEt	_	30	-1.48	-1.215	-1.249	-0.995
3	B	SPr	_	2000	-3.300	-2.465	-2.219	-2.492
4	C	Et	_	2	-0.300	-0.806	-0.956	-0.871
5	C	Benzyl	_	70	-1.850	-0.960	-1.251	-1.870
6	D	NH_2	Me	10	-1.000	-0.818	-1.170	-0.683
7	D		Me	1.5	-0.180	-0.532	-0.550	-0.437
8	D		Me	3.5	-0.540	-0.761	-0.55.	-0.491
9	D		Me	5	-0.700	-0.401	-0.550	-0.878
10	D		Et	20	-0.230	-0.402	-0.257	-0.356

Table 3: 10 Pharmacophore Hypotheses Generated Using 20 Training Set Molecules

Hypothesis[a] No	Total Cost	Cost Difference (Null cost – Total cost)	Error Cost	RMS	Correlation (r)	Features[b]
01	100.626	49.312	77.643	1.018	0.940	A H R R
02	102.325	47.613	81.886	1.208	0.909	A H H R
03	102.822	47.116	81.212	1.180	0.915	A H R R
04	103.198	46.74	82.407	1.230	0.906	A H R R
05	103.805	46.133	83.487	1.273	0.898	A H H R

06	103.839	46.099	83.977	1.292	0.894	A H H R
07	104.005	45.933	84.916	1.328	0.887	A H H R
08	104.93	45.008	84.707	1.320	0.889	A H H R
09	104.954	44.984	86.295	1.379	0.877	A H H H R
10	105.385	44.553	83.560	1.276	0.900	A A H R

a. Null cost = 149.938, Fixed cost = 85.9304, Configuration = 17.5334, Weight = 1.963, b. A, Hydrogen Bond Acceptor; H, Hydrophobic Aliphatic; R, Ring Aromatic.

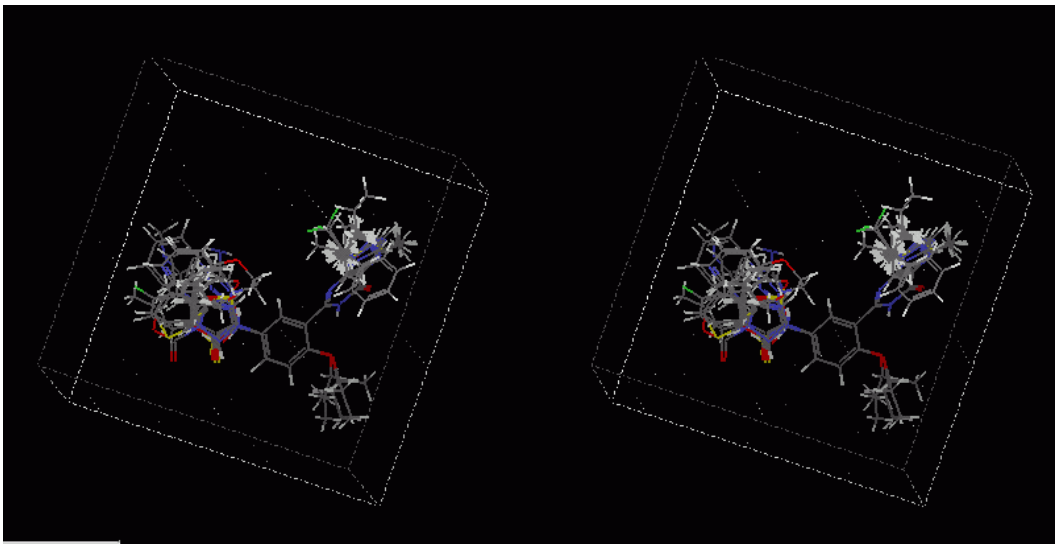


Fig 1: Stereo view of rectangular molecular field surrounding aligned molecules. Some of the field descriptors, which are involved in the equation, are indicated. Correlation of MFA (0.844)

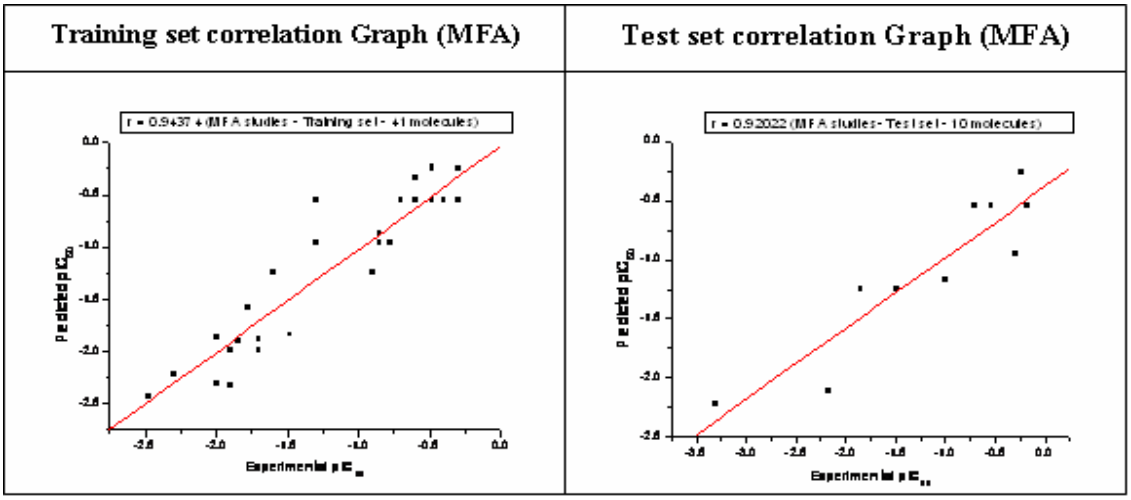


Fig 2: Stereo view of the receptor surface which represents the vitural active site. Some of the RSA descriptors that constitute the equation are labeled. Correlation of RSA (0.853).

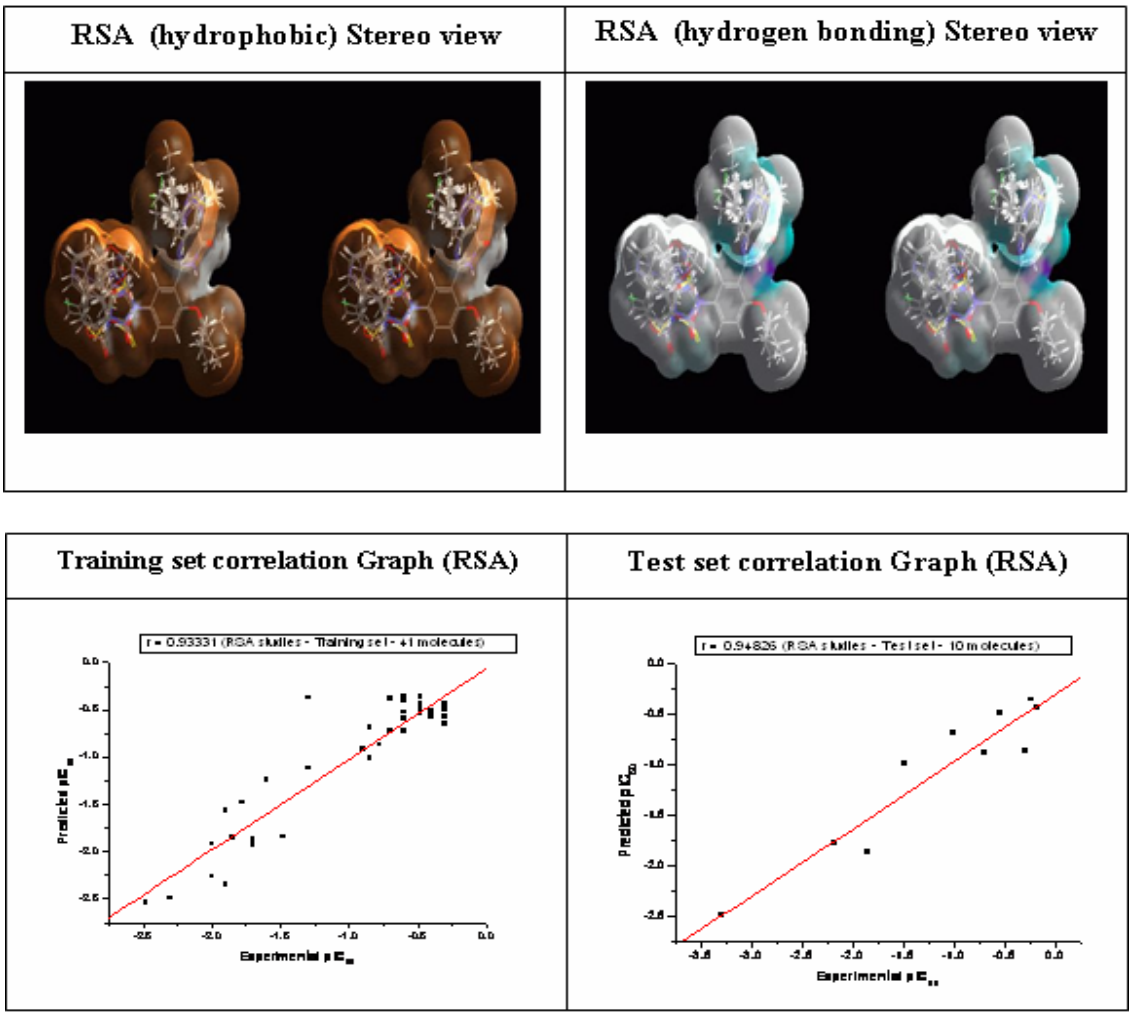
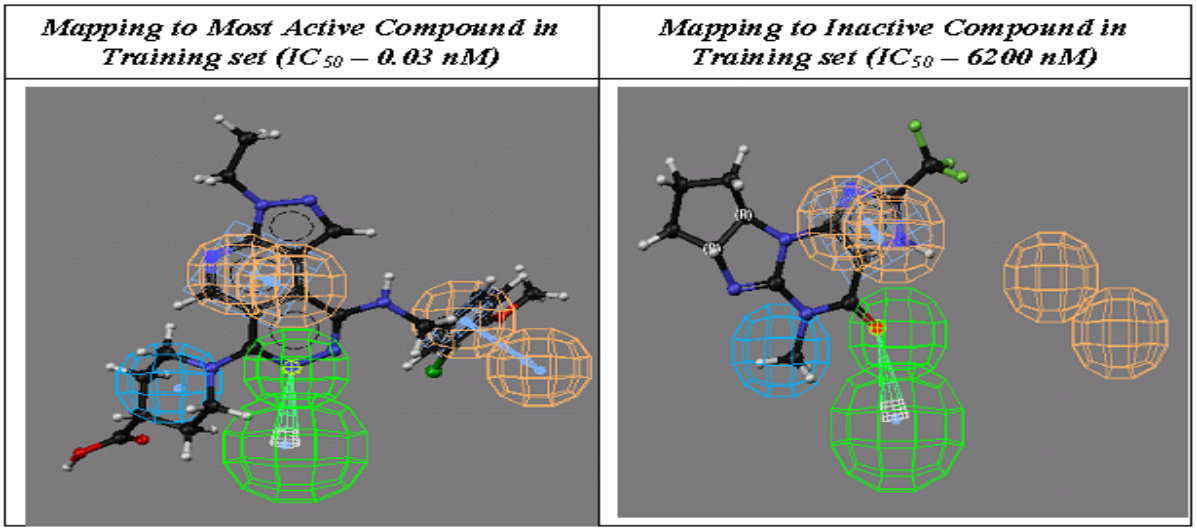
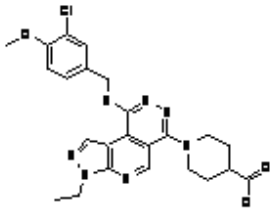
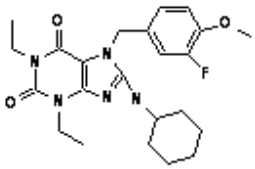
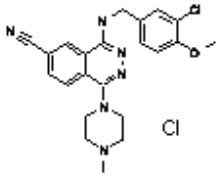
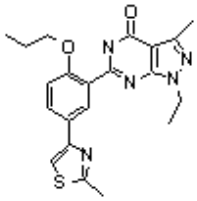
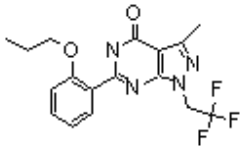
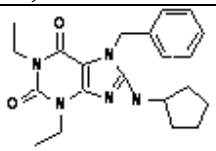
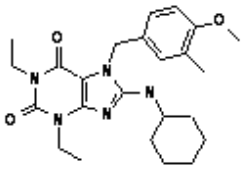
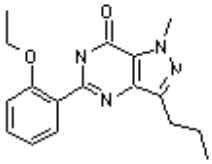
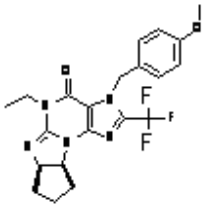
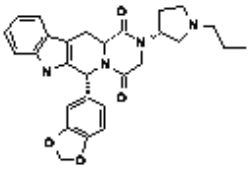
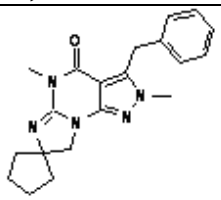
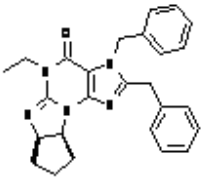
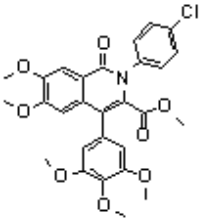
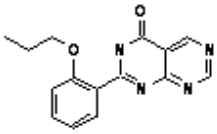
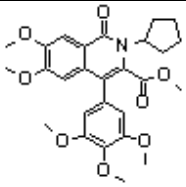
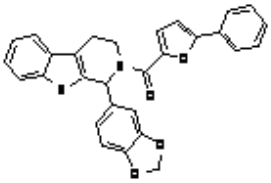
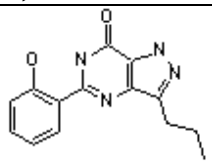
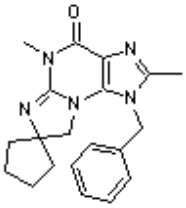
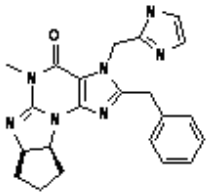
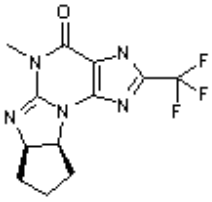


Fig 3: Pharmacophore Mapping



Chemical Structures of the 20 Training Set Molecules Applied to HypoGen Pharmacophore Generation (PDE-5 Activities Are Given as IC50 Values)

20 Training set molecules used for validation studies

			
1, IC50 0.03 nM	2, IC50 0.3 nM	3, IC50 0.95 nM	4, IC50 3 nM
			
5, IC50 7 nM	6, IC50 10 nM	7, IC50 19 nM	8, IC50 27 nM
			
9, IC50 48 nM	10, IC50 57 nM	11, IC50 75 nM	12, IC50 130 nM
			
13, IC50 170 nM	14, IC50 300 nM	15, IC50 580 nM	16, IC50 750 nM
			
17, IC50 1000 nM	18, IC50 2900 nM	19, IC50 4000 nM	20, IC50 6200 nM

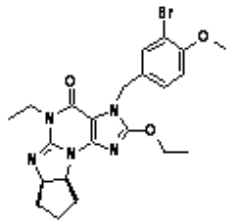
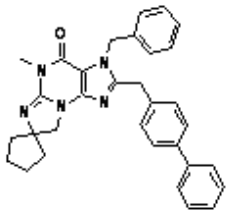
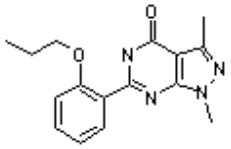
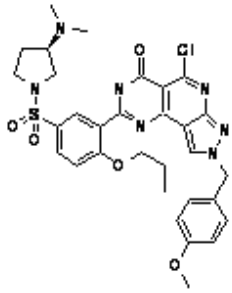
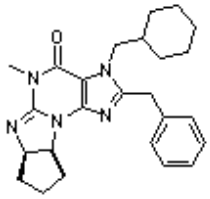
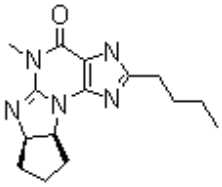
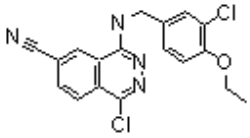
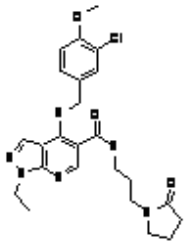
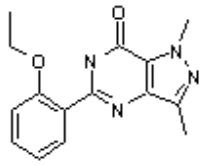
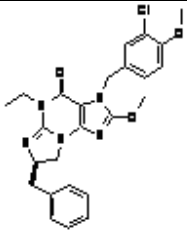
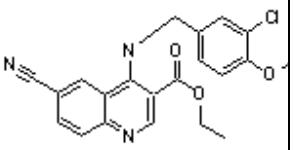
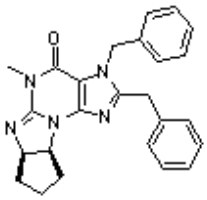
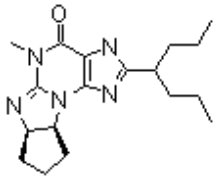
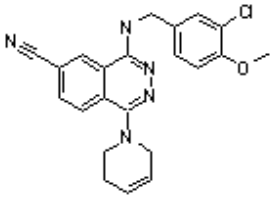
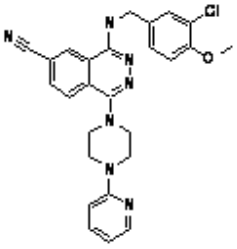
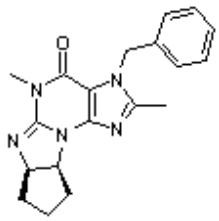
RSA Model with Hydrophobic property and Hydrogen bonding mapped onto it is shown in Fig. 2.

Statistical details of 2D, MFA, & RSA analysis were given in Table. 1

Pharmacophore Hypothesis Generation

Training set consists of 20 compounds tested against PDE-5 was used to develop Pharmacophore hypotheses.

75 Test set molecules used for validation studies

			
1, IC50 1.6 nM	2, IC50 1.6 nM	3, IC50 1.9 nM	4, IC50 2.2 nM
			
5, IC50 3.1 nM	6, IC50 4 nM	7, IC50 4.4 nM	8, IC50 5.3 nM
			
9, IC50 5.4 nM	10, IC50 6.8 nM	11, IC50 8 nM	12, IC50 11 nM
			
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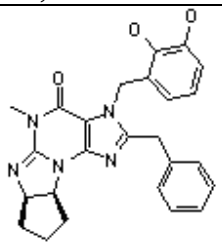
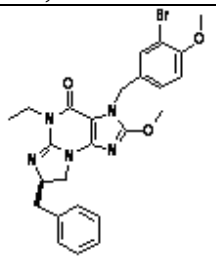
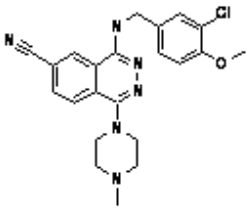
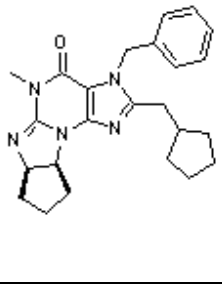
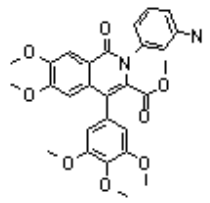
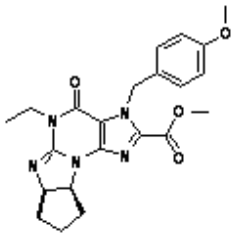
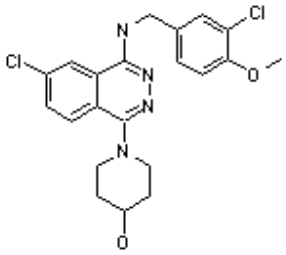
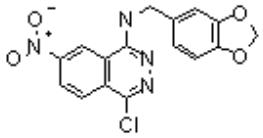
			
17, IC50 19 nM	18, IC50 20 nM	19, IC50 22 nM	20, IC50 30 nM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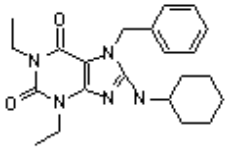	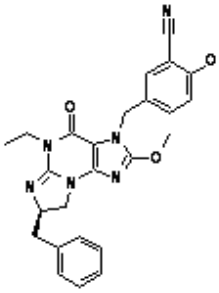	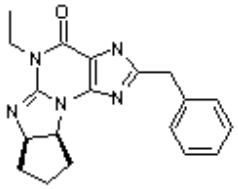	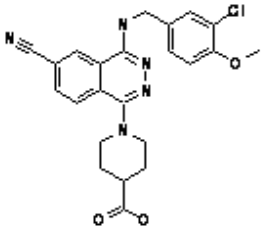
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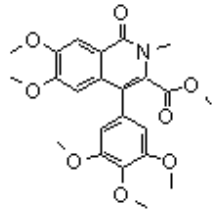
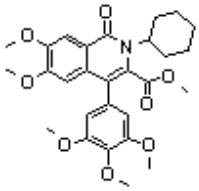
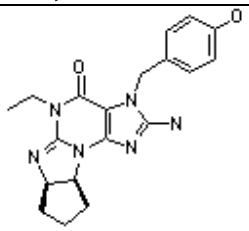
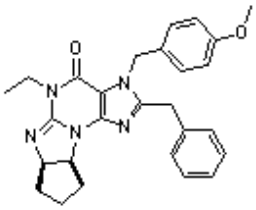
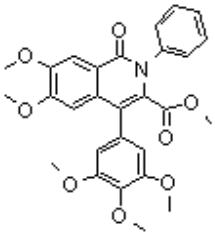
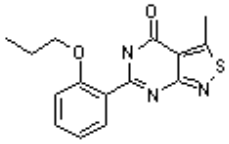
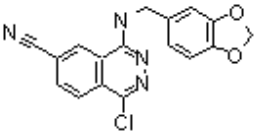
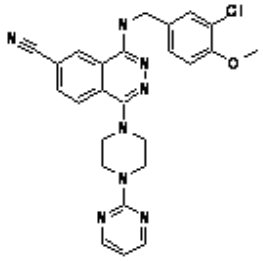
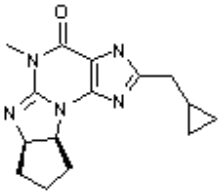
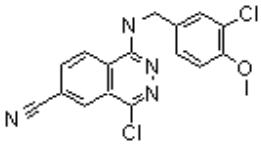
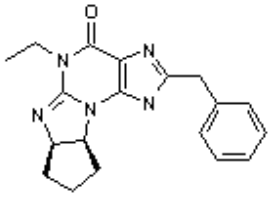
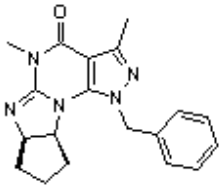
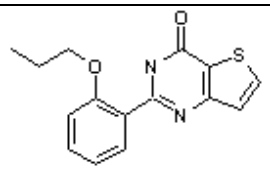
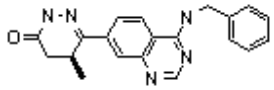
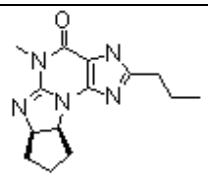
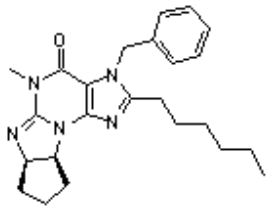
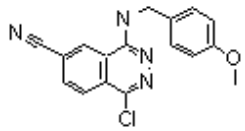
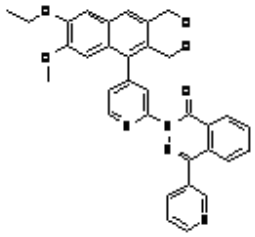
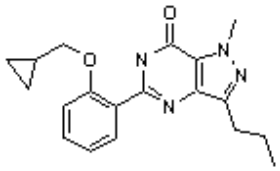
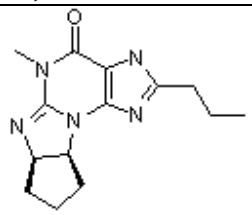
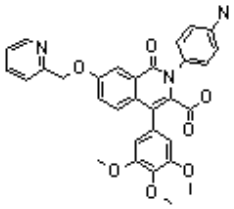
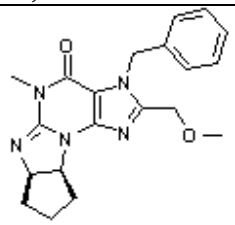
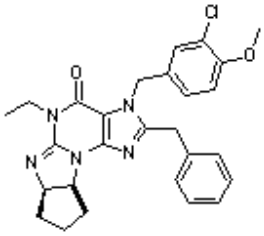
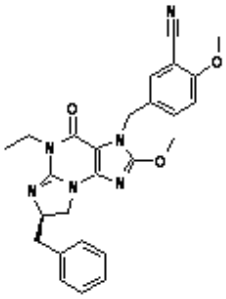
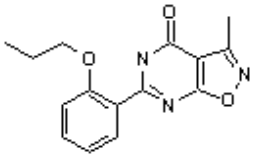
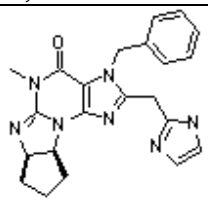
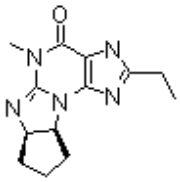
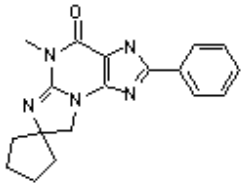
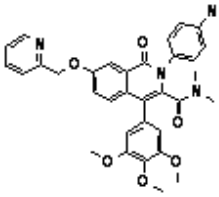
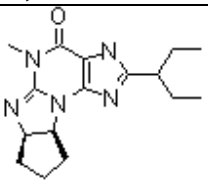
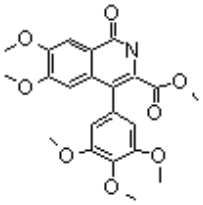
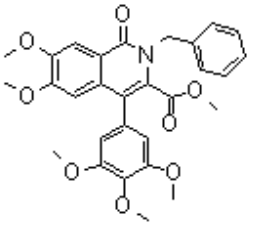
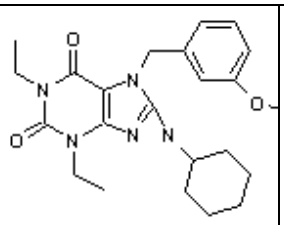
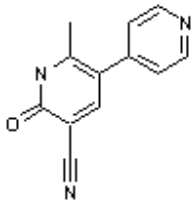
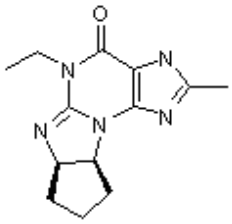
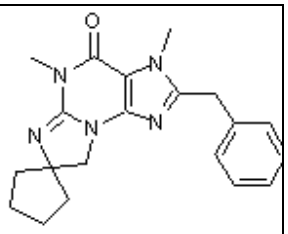
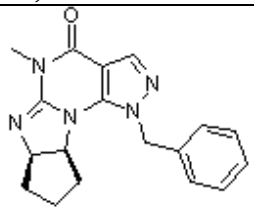
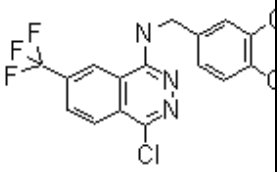
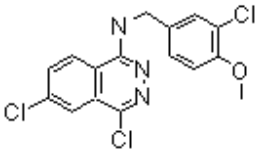
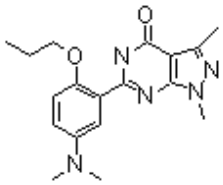
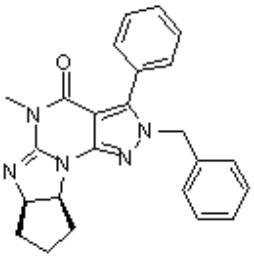
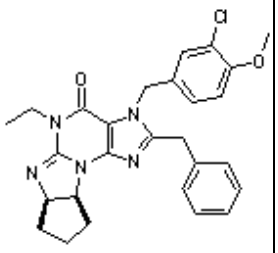
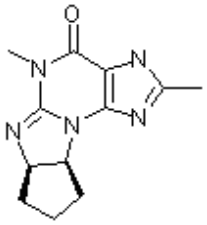
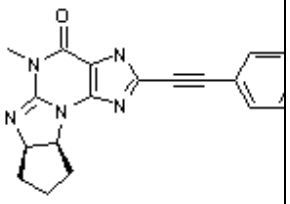
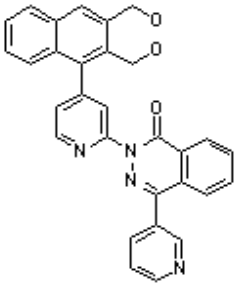
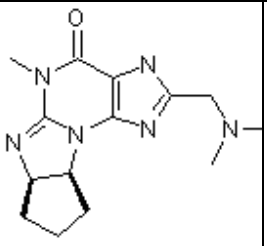
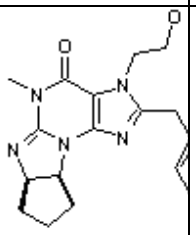
			
29, IC50 100 nM	30, IC50 100 nM	31, IC50 100 nM	32, IC50 110 nM
			
33, IC50 140 nM	34, IC50 140 nM	35, IC50 140 nM	36, IC50 150 nM
			
37, IC50 160 nM	38, IC50 180nM	39, IC50 200 nM	40, IC50 200 nM

			
41, IC50 200 nM	42, IC50 225 nM	43, IC50 230 nM	44, IC50 230 nM
			
45, IC50 2250 nM	46, IC50 275 nM	47, IC50 330 nM	48, IC50 380 nM
			
49, IC50 610 nM	50, IC50 800 nM	51, IC50 800 nM	52, IC50 800 nM
			
53, IC50 810 nM	54, IC50 900 nM	55, IC50 960 nM	56, IC50 1000 nM
			
57, IC50 1000 nM	58, IC50 1000 nM	59, IC50 1000 nM	60, IC50 1200 nM

			
61, IC50 1400 nM	62, IC50 01500 nM	63, IC50 1900 nM	64, IC50 2100 nM
			
65, IC50 2100 nM	66, IC50 2400 nM	67, IC50 2900 nM	68, IC50 3100 nM
			
69, IC50 3300 nM	70, IC50 4500 nM	71, IC50 5000 nM	72, IC50 6000 nM
			
73, IC50 7500 nM	74, IC50 10000 nM	75, IC50 1000 nM	

A total of 10 hypotheses were generated and its different cost values, correlation coefficients (r), RMS deviations, and pharmacophore feature definitions are listed in Table 3. For the training set the accuracy in predicting active and inactive compounds was 90%. The selected pharmacophore hypothesis yielded a RMS deviation of 1.018 and a correlation coefficient of 0.940 with a cost difference of 49.312. The best pharmacophore model was validated on 75 test molecules to give correlation value of 0.898. For the test set, the accuracy in predicting active compounds was greater than 10%, while 14% and 6% representing both false positive and negative respectively. The mapping of Hypothesis1 model onto an active and inactive training set Compound (IC50 = 0.03 nM and 6200 nM respectively) is shown in Fig 3.

Conclusion:

The results from these QSAR analyses provide a useful insight into the structural and electrostatic requirements for binding of a ligand to the PDE-5 receptor and these derivatives 2D, MFA and RSA could provides us useful information for developing extremely potent ligands leading to potential PDE-5 inhibitors. In 2D QSAR, the shape of the molecule is more important in relation to biological activity. In 3D QSAR, MFA studies shows that steric buck groups seem to play a crucial role on preferred locations on the analogs, such that it improves the activity and RSA shows the role of vander waals and electrostatic interactions. Further, the knowledge of this four-feature pharmacophore hypothesis for PDE-5 inhibitors can be very useful for virtual screening to design more potent lead moieties for the treatment of various types of Erectile Dysfunction.

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